Microbe Hunters: Searching for Anammox Bacteria in the Tennessee Aquarium.

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Abstract

Ammonium and nitrite are toxic metabolic waste products generated by aquatic macroorganisms. They are of particular concern in closed systems, such as commercial aquaria. Typically, biological filtration systems are employed to regulate levels of toxic N species as they are more cost-efficient compared to water removal and replacement. Microbial communities that reside in these systems play vital roles in transformation of toxic N species. Commonly, nitrite and ammonium are converted into nitrate via nitrification. However, even nitrate is toxic at higher concentrations. Bacteria belonging to the phylum Planctomycetes can transform ammonium and nitrite to N2 via anaerobic ammonium oxidation (anammox). In this study, we are investigating the presence and role of anammox bacteria in multiple tanks at the Tennessee Aquarium in Chattanooga, TN. DNA was extracted from water and filtration systems of four different aquaria. Metagenomic analyses, looking for the presence of genes diagnostic of the anammox reaction, were performed and no known annamox pathway-specific genes were identified. Given the typically low representation of Planctomycetes in microbial communities, a nested PCR approach targeting Planctomycete-specific 16S rRNA genes was used to enrich for and identify organisms capable of anammox. Analysis using this targeted PCR approach is ongoing.

Introduction

- In closed aquatic systems, toxic nitrogen species such as ammonium, nitrate, and nitrite accumulate and negatively impact the health of the aquatic macroorganisms in the system[1,2].
- Employment of microbial communities that can transform ammonium and nitrite has already been integrated into the Tennessee Aquarium via sulfur-driven denitrification (SDN).
- Sulfur-driven denitrification requires carbon to catalyze the reaction[3].
- A calcium by-product has additionally been found to occur with SDN in the Tennessee Aquarium, which is difficult to remove and potentially toxic to the system.

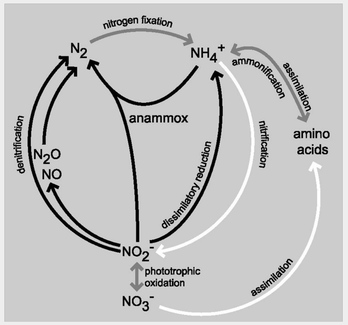

Figure 1. Representation of the Nitrogen cycle. Figure taken from Niftrik and Jetten. 2012[4].

- Planctomycetes capable of performing anammox are a possible new solution to remove toxic N species without a carbon catalyzer or toxic by-products.
- Anammox has only been demonstrated in a subgroup of bacteria within the Planctomycetes.
- General and targeted approaches are necessary to establish presence of anammox-performing Planctomycetes.

16S rRNA Analysis

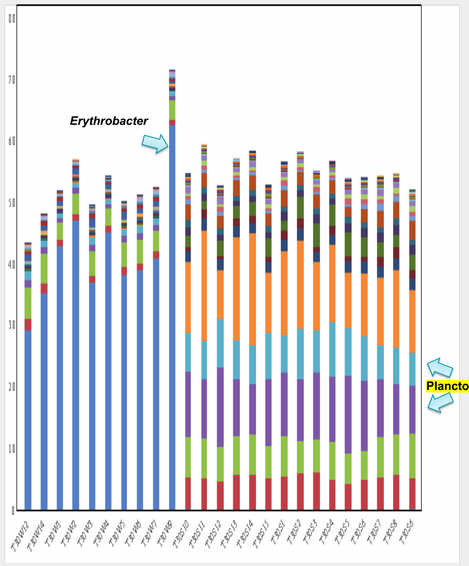

Figure 2. Percent abundance of microbial genera in the temperate marine tank (T30). Planctomycetes, which are the only phylum of bacteria capable of performing anammox, are present in this system.

Metagenomic Analysis

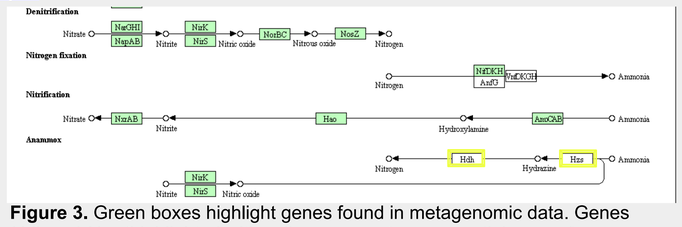

Figure 3. Green boxes highlight genes found in metagenomic data. Genes *Hdh* and *Hzs* (highlighted with yellow squares) are essential genes for anammox metabolism[5]. Both genes are not present in metagenomic analysis. This could be due to low coverage of the metagenomic data.

Nested PCR and Sequencing

- A nested PCR protocol was established to target and amplify 16S rRNA gene sequences from anammox capable Planctomycetes at the Tennessee Aquarium.

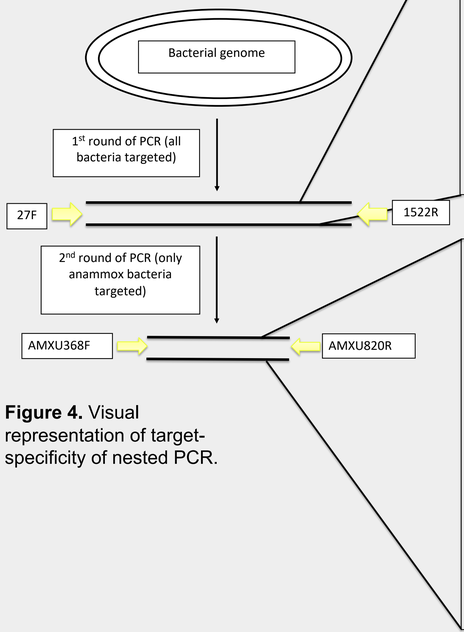

Figure 4. Visual representation of target-specificity of nested PCR.

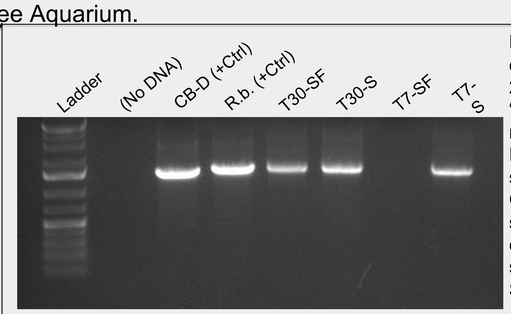

Figure 5. First round of nested PCR with 27F and 1522R "universal" 16S rRNA gene primers. Expected product size of ~1500 bp. CB-D and R.b. served as positive controls. Freshwater sand sample (T7-SF) did not amplify.

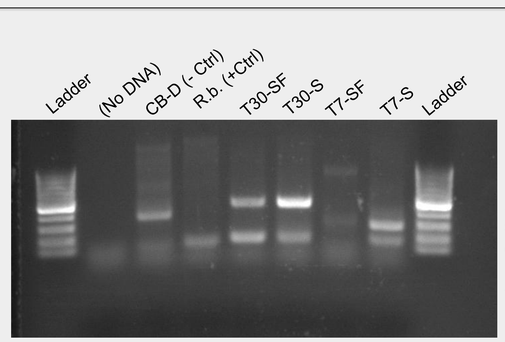

Figure 6. Second round of nested PCR with AMXU (anammox specific primer set) expected product size of ~500 bp. CB-D *(Sulfitobacter)* and *R. baltica* (R.b.) served as negative controls. PCR product amplified from the T30-SF (sand filtration) and S (water) sample is the correct size.

Conclusions

- The presence of Planctomycete 16S rRNA gene sequences in the T30 marine tank suggests the potential for anammox metabolism.
- Metagenomic analysis did not reveal presence of genes essential for anammox.
  - However, this could be due to low sequencing coverage in our metagenomic analysis and low relative abundances of planctomycetes in our samples.
- The metagenomic analysis was only representative of a small portion of the aquaria samples.
  - Nested PCR approaches, specific for anammox metabolism planctomycete bacteria, are currently being optimized.

Future Directions

- Complete optimization of nested PCR protocol to first amplify Planctomycete-specific genome region, not just 16S rRNA gene.
- Acquire known anammox-performing Planctomycetes to serve as a positive control for nested *rrn* operon PCRs.
- Develop a small scale bioreactor to grow known anammox Planctomycetes in a laboratory setting.

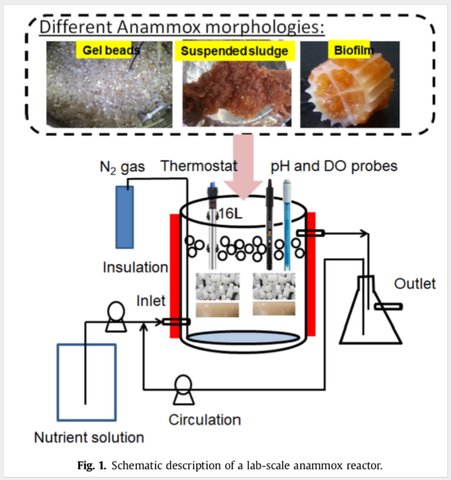

Figure 7. Schematic of anaerobic bioreactor to encourage the growth of anammox planctomycetes. Figure taken from Wu et al. 2018[5].

Acknowledgements

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References

1. Carmargo et al. (2005). "Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates". *Chemosphere*.
2. Ip and Chew. (2010). "Ammonia production, excretion, toxicity, and defense in fish: a review". *Frontiers in Physiology*.
3. Lau et al. (2016). "An oligotrophic deep-subsurface community dependent on syntropy is dominated by sulfur-driven autotrophic denitrifiers". *PNAS*.